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The Culture of Larval Penaeid Shrimp[1]

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ABSTRACT

Large numbers of penaeid shrimp were reared to postlarvae from eggs spawned in the laboratory. Rearing containers were four 1,040-liter tanks and one 1,890-liter tank.

Temperature affected the rate of development through the larval stages. In water of 30 ppt (parts per thousand) salinity, the average time required for larvae of brown shrimp to reach first postlarval stage was 17 days at 24 C, 12.5 days at 28 C, and 11 days at 32 C. Survival of nauplii was best at 24 C, and as the shrimp became protozoeae and myses, survival usually increased with an incease in temperature.

Larvae did not survive at salinities above 35 ppt or below 27 ppt.

Algae tested for shrimp food were: *Cyclotella nana, Isochrysis galbana, Skeletonema costatum, Thalassiosira* sp., and a mixture of all four types. *Isochrysis* appeared to be the least suitable food, and the mixed algae and *Thalassiosira* probably were the best.

INTRODUCTION

Over the past few years interest has grown in pond culture of shrimp in the United States. Progress in research has been limited, however, because of the difficulties in obtaining young shrimp with which to stock ponds. Postlarvae of white shrimp (*Penaeus setiferus*), brown shrimp (*P. a. aztecus*), and pink shrimp (*P. d. duorarum*) enter the estuaries in large numbers, but their abundance is seasonal and it is frequently impossible to obtain postlarvae when they are needed. At time when postlarvae are available, many other species are commonly taken with the shrimp, and it is laborious and time-consuming to separate them.

The logical alternative is to rear postlarvae from eggs. In Japan, larval shrimp have been cultured for many years and the procedures have been described in detail (Hudinaga, 1942; Hudinaga and Miyamura, 1962; Hudinaga and Kittaka, 1966, 1967; Fujinaga, in press). Most other attempts at culturing large numbers of larvae have been unsuccessful. After several years of research (Cook and Murphy, 1966; Cook, in press) we have developed a method of rearing relatively large numbers of penaeids. The method is similar to that used by Hudinaga in his early work, but we have made modifications that increase the yield. The yield of postlarvae per volume of water, however, must be increased even further before commercial production can be considered. Our method, nevertheless, should enable researchers to grow, at a relatively low cost, enough postlarvae for pond culture experiments.

We have cultured six species of shrimp, including the commercially important brown, white, and pink shrimp. The work was done in Galveston, Texas, but we have evidence that the method can be adapted for use in other areas. Members of this Laboratory's staff in Miami, Florida, have recently succeeded in culturing a large number of pink shrimp larvae to postlarvae by the methods described here.

METHODS

Larval Culture

Shrimp native to the Gulf of Mexico have not been reared to sexual maturity under controlled conditions; consequently, we must still depend on wild stocks for a supply of females in spawning condition. These shrimp are taken on the offshore spawning grounds with a commercial 14-m otter trawl. The length of tow is usually between 10 and 30 minutes, depending on the amount of fish and trash taken with the shrimp. The catch is deposited directly on deck, and the ripe females are sorted immediately and placed in a tank through which water is circulated continuously.

Ripe penaeid shrimp can be identified easily. Their eggs are carried internally, and when they are in spawning condition the two lobes of the ovary which extend down the

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abdomen are dark olive-green in brown and pink shrimp, and olive-brown in white shrimp. It is not necessary to collect males because the females are impregnated with a spermatophore and store the sperm until it is released during spawning.

The shrimp are transported from the ship to the laboratory in 75-liter plastic barrels. At the laboratory they are placed in 1,890-liter or 1,040-liter round polyethylene tanks (Figure 1) containing water that has been passed through a 5μ cellulose filter. The 1,890-liter tanks are 150 cm high and 142 cm in diameter, and the 1,040-liter tanks are 123 cm high and 121 cm in diameter. Before the shrimp are placed in the tanks, low-salinity water is raised to at least 27 ppt (parts per thousand) by the addition of Instant Ocean Sea Salts,[2] and the sodium salt of ETDA ([ethylenedinitrilo]-tetracetic acid) is added to the water at a rate of 1 gram per 100 liters.

Water in the tanks is recirculated continuously through a crushed-oyster-shell filter and is aerated vigorously from lead-weighted air stones suspended from the sides of the tanks. To prevent eggs and larvae from being pumped into the filters, bags of nylon plankton mesh (0.12-mm mesh opening) are attached by elastic bands to a 13-cm diameter, 1.3-cm thick polyvinyl chloride plastic plate through which the outlet hose is inserted. A tube of heavy plastic mesh is placed inside each bag to keep it from collapsing. The bags have a surface area of 864 cm^2. Filtering of the water but not the aeration is stopped at the first protozoeal stage when the first food is added.

It is usually not necessary to change water in the tank during the term of the culture. If, however, dead larvae are seen in a tank, or the water becomes fouled from overfeeding, one-half of the water in the tank is changed daily until the situation is corrected.

One or two adult shrimp are placed in each tank. Only about one-third of the shrimp we isolate spawn viable eggs—usually during the first night after they are brought to the laboratory. The rest either deposit a single mass of eggs which fail to develop or they resorb their eggs within a few days. After the shrimp spawn, they are removed from the tank to prevent them from eating the eggs.

The eggs hatch 12 to 16 hr after spawning. The larvae do not feed during the first larval (nauplial) stage. Feeding begins at the second larval (protozoeal) stage. During this stage the larvae are fed algae. *Skeletonema costatum*, *Thalassiosira* sp., *Cyclotella nana*, *Phaeodactylum* sp., *Dunaliella* sp., *Gymnodinium splendens*, and *Exuviella* sp. have all been used successfully as food for protozoeae. Newly hatched brine shrimp (*Artemia* sp.) are supplied for food during the third stage (mysis). The addition of algae is stopped 1 day after the introduction of brine shrimp.

Shrimp larvae are fed once a day. When algae are fed, a portion of the water in each tank is removed and replaced with algal culture. Our feeding rate is 1 liter of algae having a density equivalent to 2×10^5 cells of *Skeletonema* per ml for every 15 liters in the tank.

Algal Culture

The major remaining problem is that of culturing enough algae to feed large numbers of shrimp larvae. We are growing the algae in enriched sea water, but because the composition of sea water varies during the year, it is difficult to determine which additives support the best growth of algae.

We are presently investigating three methods of algal culture. In the first, sea water is strained through a 0.12-mm-mesh plankton net to remove the zooplankton. The sea water is then enriched with 1 gram of ferric sequestrine, 1 gram of KNO_3, 0.5 gram of Na_2HPO_4, and 0.5 gram of Na_2SiO_3 for each 100 liters of water. A dense growth of phytoplankton usually appears within 3 or 4 days. One problem with this type of culture is that the species of alga that becomes dominant is not always suitable as food for the larval shrimp. Also, ciliates, copepods, or similar organisms sometimes contaminate the cultures; these cultures must be discarded.

In the second culture method, sea water is passed through an 0.8μ membrane filter. The water in each tank is then inoculated from unialgal cultures of several species of diatoms

[2] Trade names referred to in this publication do not imply endorsement of commrecial products.

and enriched as in the first method. By isolating diatoms at different times of the year we will eventually have 8 or 10 kinds with differing physiological requirements. We anticipate that the water at any given time will support dense growths of one of these forms.

In the third method, several species of diatoms are cultured in 75-ml test tubes to which different enrichments are added singly and in combination. The additives used are: KNO_3, Na_2HPO_4, Fe, EDTA, B_{12}, and Gates and Wilson's (1960) trace metal mixture. We can usually determine within 3 days which diatom is growing best and the nutrient mixture it requires. This combination of alga and enrichment is then used for mass culture.

Our large algal cultures are grown in a greenhouse in open tanks that contain 640 liters of sea water. The cultures are open to the air and become contaminated after 8 to 10 days. As a result, we alternate cultures, starting a new one every 4 or 5 days.

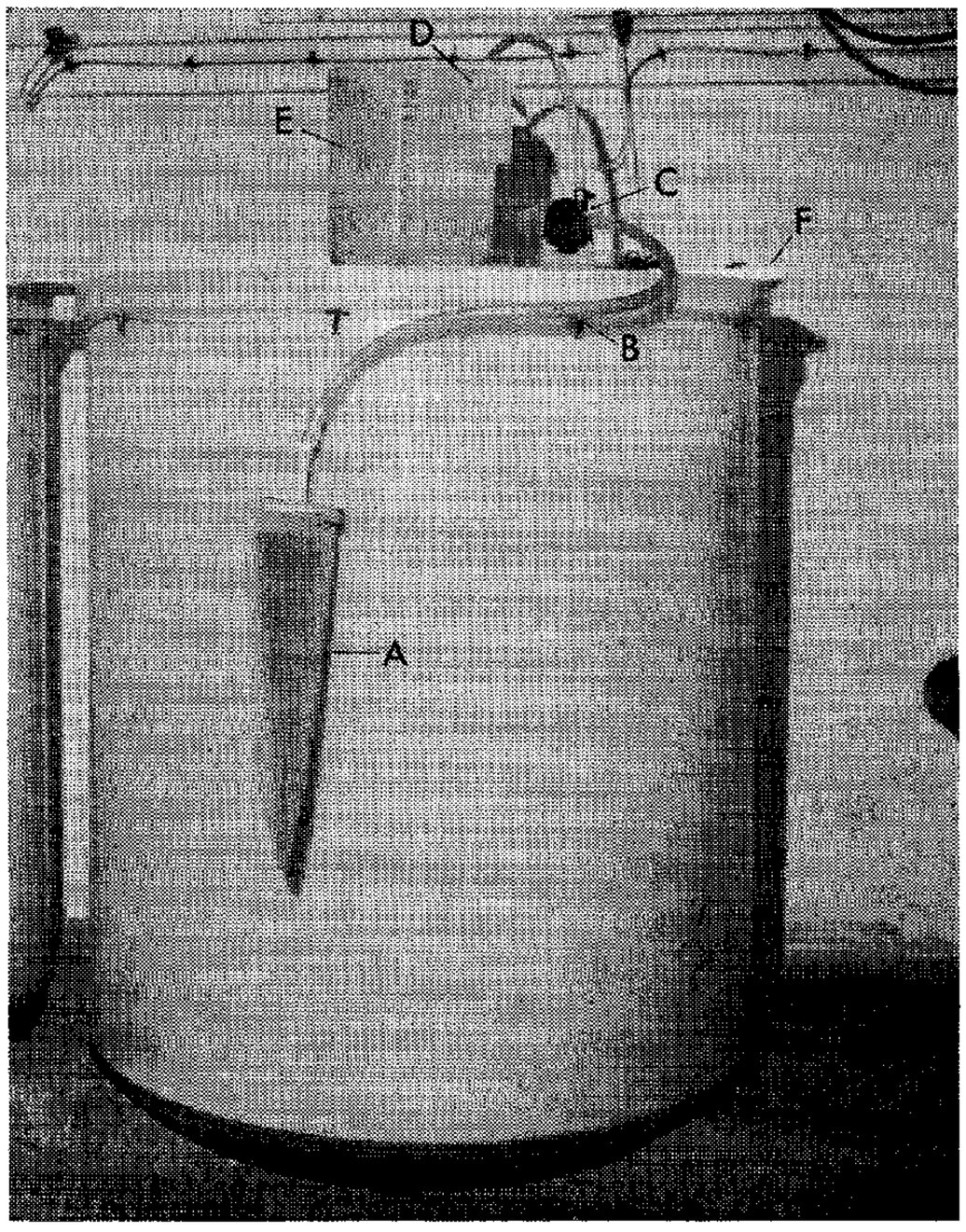

FIGURE 1.—Tank (1,040-liter) and equipment used in the culture of larval shrimp: A, inlet screen; B, air valve; C, pump; D, water baffle; E, filter; F, stand.

LIMITING FACTORS

Density of Larvae

The density of larvae that can be cultured in a given amount of water appears to depend on several factors of which food, temperature, and salinity are probably the most important. For our estimates of optimum larval density, we have used the inverted 19-liter polyurethane carboys described by Cook (in press). As many as 4,000 shrimp have been reared from nauplii to postlarvae in one of these carboys holding 15 liters of sea water. The average yield per carboy stocked with 4,000 nauplii (266 per liter) has been 2,000 postlarvae (133 per liter). Consequently, we now adjust the number of nauplii in the large tanks to 266 or less per liter as soon as possible after hatching. Our largest yield in a 940-liter tank has been 65,000, or 64 postlarvae per liter in a tank that was stocked with 92 nauplii per liter. When we have more information on how other factors affect mortality, this number can probably be increased.

Temperature

Temperature seems to affect the rate of development of the larval shrimp. No larvae have completed development at temperatures below 24 C. The rate of larval development increased with increasing temperature up to 32 C—the highest temperature tested. In water of 30 ppt salinity, the average time required for larvae of brown shrimp to reach the first postlarval stage was 17 days at 24 C, 12.5 days at 28 C, and 11 days at 32 C.

Larvae of brown shrimp hatched in water of 29 ppt salinity and 27 C temperature were held in water of 30 ppt salinity at four temperatures (20, 24, 28, and 32 C) to determine the effects of temperature on survival. At the start of each larval stage, 120 larvae (20 in each of six 250-ml beakers containing 150 ml of sea water) were held at each temperature. The stage of development and the number of larvae surviving were recorded daily. Survival of nauplii was best at 24 C and as they became older, i.e., protozoeae and myses, survival usually increased with an increase in temperature (Table 1).

Salinity

No extensive experiments have been performed to determine the effects of salinity on

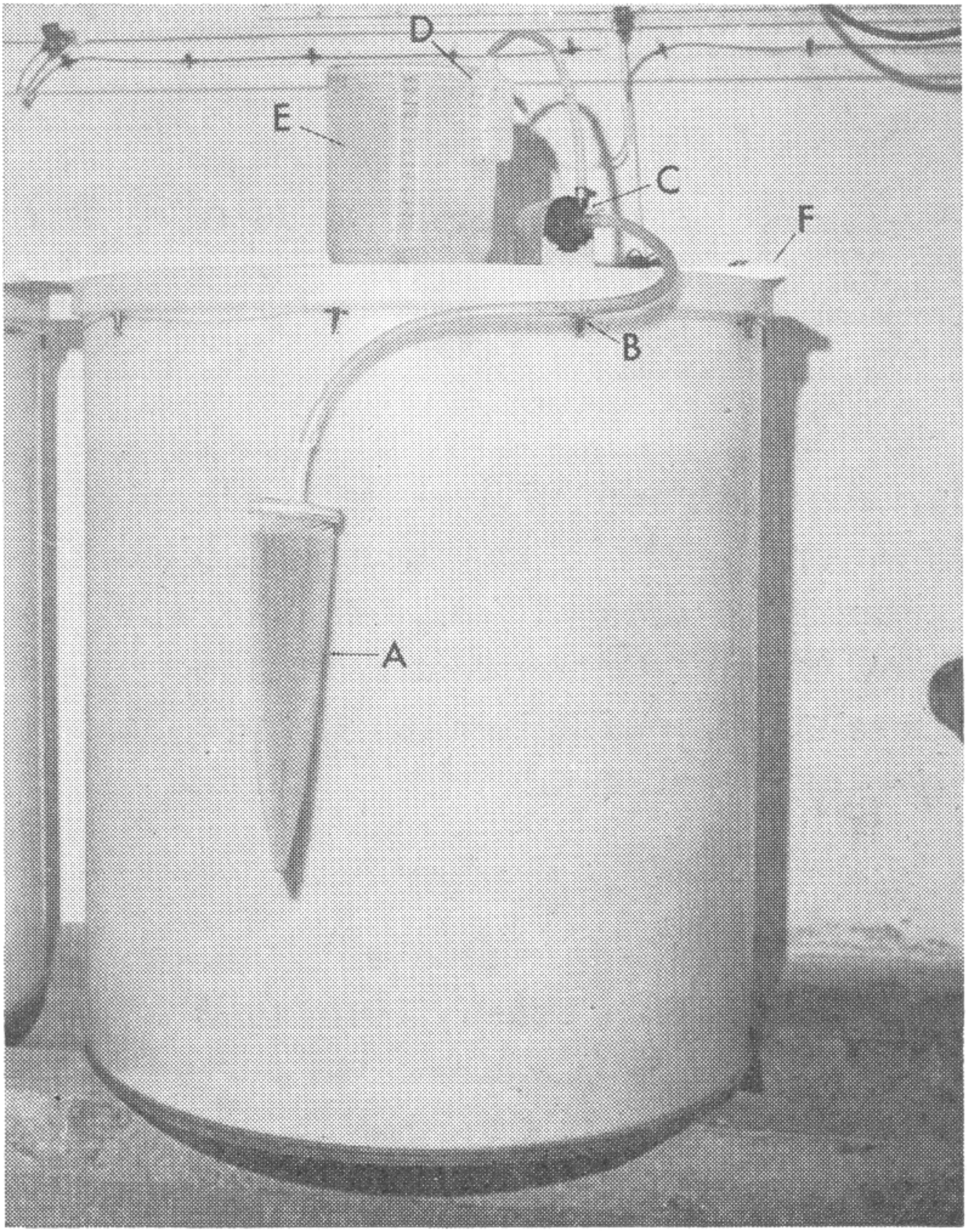

FIGURE 1.—Tank (1,040-liter) and equipment used in the culture of larval shrimp: A, inlet screen; B, air valve; C, pump; D, water baffle; E, filter; F, stand.

TABLE 1.—*Percentage survival of the larvae of brown shrimp held at four temperatures*

Stage of development	Temperature			
	20° C	24° C	28° C	32° C
Nauplius	7	84	44	28
Protozoea	0	63	73	68
Mysis	88	93	94	98

larval growth and survival. In our large tanks, however, hatching has been inhibited, and larvae have died at salinities above 35 and below 27 ppt. Consequently, we add artificial sea salts to low-salinity water to raise the salinity to at least 27 ppt.

Feeding

Our method of feeding algae in the large tanks produces a density equivalent to 10 to 15×10^3 cells of *Skeletonema* per ml. Hudinaga and Miyamura (1962) stated that from 5 to 10×10^3 cells of *Skeletonema* per ml is best for protozoeae of *P. japonicus*.

We conducted one experiment with protozoeae of *P. a. aztecus* to determine the lowest concentration of algae that could be used. Larvae were held in 250-ml beakers at an initial concentration equal to 133 per liter. Algae were added once a day, and the beakers were agitated to keep the food in suspension. *Cyclotella nana, Isochrysis galbana, Skeletonema costatum,* and *Thalassiosira* sp, as well as a mixture of the four types, were used as food. *Skeletonema* was fed at rates of 500, 1,000, and 1,500 cells per ml. Numbers of the other algae used were adjusted to concentrations giving cell volumes equal to those of the *Skeletonema*. Even the highest concentrations of algae were apparently too low, and all larvae died. We were, however, able to draw some tentative conclusions as to the relative value of the different algae from the lengths of time the larvae survived and the stage of development attained (Table 2). *Isochrysis* appeared to be the least suitable food, and the mixed algae and *Thalassiosira* probably were the best.

In a similar experiment, myses of *P. a. aztecus* (initial density, 150 per liter) were fed at rates of three, four, and five *Artemia* nauplii per ml of sea water per day. At the two highest feeding rates, the shrimp consumed more *Artemia* but had poorer survival to first postlarvae than at the lowest rate. Those fed at a rate of 3 Artemia per ml had a survival rate of 82% and consumed an average of 18 *Artemia* per day; those fed at rates of four and five per ml had survival rates of 32% and 65%, while consuming 22% and 69% more *Artemia* per day. The lots showed no difference in the time taken to reach the first postlarval stage.

TABLE 2.—*Survival and stage of development attained by brown shrimp protozoeae fed selected algae*

Algae fed	Number of days survived	Stage of development attained
Isochrysis galbana	2	Protozoea I
Cyclotella nana	3	Protozoea I
Skeletonema costatum	4	Protozoea I
Mixture	4	Protozoea II
Thalassiosira sp.	5	Protozoea II

We have just begun to investigate the physiological requirements and tolerances of larval shrimp, and we think we have some interesting leads. We believe the yield of postlarvae per volume of water should increase significantly as we learn more about such factors as the temperature-salinity relationship and feeding rates in relation to larval density.

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